2734-Pos Board B504
The Asymmetric Lipid Bilayer Revealed Sidedness of the Effective Phospholipids on the Single-Channel Properties of the KcsA Potassium Channel
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Function of ion channels is modulated by the composition of lipid bilayer. Some ion channels lose their activity when they are reconstituted into alien membrane environment. Thus, membrane lipids act as a co-factor for maintaining the channel activity as in the case for other membrane proteins. For the KcsA potassium channel, an anionic phospholipid has been thought to play an essential role in its function because KcsA exhibited reduced ion conducting activity in the artificial membrane without phosphatidylglycerol (PG). Relevance of PG to the KcsA function has been further supported by crystallographic studies that revealed specific binding of PG molecules to the transmembrane region of the channel. However, little is known about the mechanism on how lipid molecules, including PG, modulate the function of KcsA channel. In this study we analyzed effects of lipids on the single-channel current properties of KcsA using artificial lipid bilayer having asymmetric lipid composition. We revealed that presence of PG in the membrane significantly increased the open probability and unitary conductance of KcsA channel. We also found that these effects were substitutable with other anionic phospholipids such as phosphatidylserine and phosphatidic acid but not by neutral or cationic phospholipids such as phosphatidylcholine or ethyl phosphatidylcholine. Furthermore, the sidedness of these anionic lipid effects was analyzed. We concluded that the binding of anionic lipids to the specific lipid-binding sites, which had been found in the KcsA crystal structure, would not be an origin of these anionic lipid effects.

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Inhibition of MthK K^+ Channels by Mg^{2+} and Polyamines: Inward Rectification with a Short Pore
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K^+ conduction through K^+-selective channels is vulnerable to blockade by divalent cations; inwardly-rectifying K^+ (Kir) channels are especially sensitive to inhibition by cytoplasmic cations such as Mg^{2+}, as well as naturally-occurring polyamines such as spermine and spermidine, and this sensitivity is critical to the function of these channels within their physiological niche. Here we report that MthK, a prokaryotic K^+ channel and relative of mammalian slo/BK channels, is highly-sensitive to inhibition by cytoplasmic Mg^{2+} and polyamines, much like mammalian Kir channels. MthK currents were inhibited in a voltage-dependent manner by spermine or spermidine, applied to the cytoplasmic side of the channel. In symmetrical 200 mM KCl, steady-state inhibition by spermine and spermidine were described by single-site models of voltage-dependent inhibition, with $K_{app}(0)$ of 48 uM and 0.68 mM, respectively. Mg^{2+} also inhibited outward current, with a $K_{app}(0)$ of 1.6 mM. Thus Mg^{2+} and polyamines might each be expected to contribute to inhibition of K+ efflux through these channels under physiological conditions. Despite the apparent affinity of Mg^{2+} and polyamines to inhibit K+ efflux through MthK channels, inhibition by each of these displayed a similarly weak voltage-dependence in symmetrical 200 mM KCl, with Z_{app}'s of ~0.7, 0.65, and 0.38 for spermine, spermidine, and Mg^{2+}, respectively. Crystallographic data suggests that K+ conduction through MthK channels occurs via a relatively short, wide pore, as opposed to the long, narrow pore of Kir channels that contains multiple K+ binding sites along its length (in single file). Thus it seems that the weak overall valences for inhibition in MthK channels vs. mammalian Kir channels is consistent with the requirement of a long, narrow pore to impart strong voltage-dependent blockade.

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Residues at the Outer Mouth of Kir1.1 Determine K-Dependent Gating
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Three residues (E132, R128 or F127) at the outer mouth of Kir1.1b directly affected inward rectifier gating by external K, independent of pH gating. Each of the individual mutations E132Q, R128Y, F127V, F127D changed the normally hyperbolic K-dependence of channel activity (K_m = 6 ± 2 mM) to a linear dependence up to 500mM. Since the single-channel currents were saturated at K > 50 mM (K_m = 8 ± 1 mM), this suggests that increases in macroscopic current reflect activation of these mutants by external K at higher than normal concentrations. These same mutations also prevented rapid recovery of channel activity following chelation of external K by 18-Crown-6 polyether. However, in divalent-free media, 2 of the mutants E132Q and R128Y (but not F127V or F127D) fully recovered after chelation of external K, consistent with previous observations that divalent removal increases the K affinity of Kir1.1. In contrast, mutating neighboring residues at the outer mouth of the channel (E92, E104 and Q133) did not alter the external K-dependence of Kir1.1b activation. These results suggest that E132, R128 and the aromatic side-chain at F127 are essential for optimal gating of Kir1.1 by external K.

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Ras-Associated (RA) Domain of Sorting Nexin 27 (SNX27) is Critical for Regulating GIRK Channels
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G-protein-gated potassium inwardly rectifying (GIRK) channels play an important role in regulating neuronal excitability in the brain. We have previously shown that trafficking and surface expression of GIRK channels are regulated by sorting nexin 27 (SNX27). SXN27 contains three domains; a phosphoinositide-binding Phox (PX) domain that targets SNX27 preferentially to early endosomes, a PDZ domain that associates directly with carboxyl-terminal tailof GIRK3 and GIRK2c subunits, and a Ras-associated RA domain that could potentially bind to small monomeric G proteins. The functional consequence of this interaction with the RA domain was unknown. Here we show for the first time that expression of a SNX27 mutant devoid of the RA domain (SNX27ΔRA) in HEK293T cells abrogates the down-regulation of baclofen-induced GIRK2c/3 currents that is typically observed with wild-type SNX27 (12.4 ± 3.6 pA/pF (SNX27 WT group) vs. 55.8 ± 8.2 pA/pF (SNX27ΔRA) vs. 47.1 ± 6.7pA/pF (control group without SNX27); mean current density ± SEM). Using bimolecular fluorescence complementation (BiFC) technique to follow the intracellular localization of GIRK2c/3 heterotetramers, we observed that SNX27 but not SNX27ΔRA increases the number of GIRK2c/3 heterotetramers in early endosomes. Moreover, a single point mutation within the RA domain (K305A) was sufficient to disrupt SNX27-dependent clustering of GIRK2c/3 heterotetramers, mimicking the functional effects of the RA deletion. Recently, it was shown that the RA domain of SNX27 interacts with H-Ras *in vitro*. We postulate that K305A disrupts RA interaction with H-Ras. In conclusion, we provide the first functional evidence that intact SNX27 RA domain and interaction with small monomeric G proteins is necessary for the regulation of GIRK channels. These new findings suggest that H-Ras signaling directly affects the magnitude of GIRK currents through regulation of SNX27.

2738-Pos Board B508
Engineering the Alcohol Pocket to Create a Chemically-Activated GIRK Channel
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G-protein gated inwardly rectifying potassium (GIRK) channels are implicated in alcohol abuse. Ethanol directly activates GIRKs through interaction with a discrete alcohol-binding pocket that we identified in the cytoplasmic domain. The structural mechanism underlying ethanol-dependent activation, however, is not well understood. Here, we hypothesized that chemically modifying the alcohol pocket with alcohol-like reagents would activate the channel. To examine this, we engineered GIRK2 containing a single cytoplasmic cysteine substitution in the βD-βE domain of the alcohol pocket; S246 is located near the edge of the pocket, and L257 is located within the pocket. All Cys substitutions were introduced into GIRK2c lacking four native Cys residues ($GIRK2c^{(cys-)}$) and transiently expressed in HEK293T cells. We studied the effect of 2-Hydroxyethyl MTS (MTS-HE, alcohol-like) and two hydrophobic MTS reagents-Benzyl-MTS (MTS-Bn), and 4-hydroxy benzyl-MTS (MTS-Y), on basal (Ba^{2+}-sensitive) and alcohol-activated GIRK currents. MTS-HE (100 μM) increased basal GIRK currents for S246C (34% ± 12%, n=7) and L257C (120% ± 24%, n=7). Similarly, MTS-Bn (10 μM) modification increased basal currents by 74% ± 18% (n=7) for S246C, and by 333% ± 31% (n=4) for L257C. MTS-Y (100 μM) increased the basal currents by 102% ± 25% (n=5) for L257C but did not affect S246C. In addition, MTS modification decreased MPD-activated current. For L257C, we confirmed that intracellular application of MTS-HE produced a concentration-dependent increase in the time course of channel activation, having a rate constant of 90.6 ± 17.2 $M^{-1}s^{-1}$ (n=11). Taken together, these data demonstrate that MTS modification of a single amino acid in the alcohol pocket can engage channel activation, and limit the access of bulkier diols (e.g., MPD). These results reveal novel mechanisms for chemical activation of GIRKs and underscore the importance of hydrophobic interactions in alcohol-mediated activation of GIRKs.